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Enhancements of Residual Soil Properties Using Vermicompost and Inoculant Strains in Low-Fertility Sandy Soil

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Abstract: In Ethiopia soil fertility and productivity has been decline due to excessive use of chemical fertilizer, top soil erosion and organic matter depletion. This laboratory and greenhouse study was conducted to isolate effective rhizobium strain and vermicompost rate to improve the crop yield and soil properties. It was carried out using Dosha variety in 2020 to 2021. The factorial combination of three rhizobium strains (non-inoculated, RS-17 and RS-1035) and four rates of vermicompost (0, 5, 10 and 15 ton ha^{-1}) treatments were arranged in complete randomized design (CRD) with three replications. The result showed that rhizobium strains and vermicompost rate had brought significant ($p<0.05$) influence on the biological and chemical properties of soils. Sole application of maximum rate of vermicompost (15 ton ha^{-1}) and rhizobium strain with RS-17 and RS-1035 significantly improve total number of microbial community. The crop inoculated by RS-17 responded the maximum number of rhizobium bacteria. Sole and combined application of 15 ton ha^{-1}vermicompost with RS-1035 or RS-17 strains produced large sized, deep red and fast growing rhizobium bacteria. On the other hand, the treatment that received combined RS-17and 15 ton ha^{-1}vermicompost was significantly improved soil's pH by 6.2%, organic carbon (37.5%), available P (13.2%) and total N (1.5%) as compared with the control treatment. Moreover, it had the maximum microbial population (1.33×108cfu/g) and out of this 7.6×105cfu/g rhizobia population. However, to forward a compressive recommendation the experiment should be supported by field studies on different area soils.

Keywords: Soil Fertility, Chemical Fertilizers, Organic Matter, Rhizobium Strain, Vermicompost

1. Introduction

The Rhizosphere is a narrow zone of soil which influenced by root exudates and inhabited by most microorganisms such bacteria, fungi and other beneficial microorganisms that improve soil health and ecosystem functioning [32]. Microorganisms in the rhizospher play a significant role in regulating the dynamics of organic matter decomposition and the availability of plant nutrients such as N, P and S [5]. Gram-negative soil bacteria of the genus Rhizobium play a very important role in agriculture [35], in stimulating the production of phytohormones, siderophores, and hydrogen cyanide as well as microbial diversity and structure, potentially enhancing plant growth-promoting rhizobacteria [2, 32]. Bio-fertilizes especially rhizobium bacteria increased availability of plant nutrients by improving soil pH in to neutral conditions [24, 30].

The earthworms' casting is nutritive organic manure rich in humus, NPK, micronutrients, beneficial microbes, antibiotics, enzymes, growth hormones [4]. During vermicomposting, earthworms physically fragment the substrate leading to new nutrient pools and large surface area which provides nutrients in forms that are readily taken up by the plants such as nitrates, exchangeable P, and soluble K, Ca, and Mg [23]. Application of vermicompost built-up soil organic carbon, improve nutrient status, microbial activities, microbial biomass carbon and enzymatic activities [10, 18]. In addition, vermicompost are rich in microbial populations and diversity, particularly fungi, bacteria and actinomycetes [10].

Manipulation of the rhizosphere is a key mechanism for solving critical issues facing the planet, including agricultural and forest sustainability, improving water quality, mitigation of climate change, and preservation of biodiversity [37, 41]. However, to improve crop yield, currently most Ethiopian farmers used to apply huge chemical fertilizer rather than incorporating organic sources of fertilizers. This action leads to the distraction of microorganisms in the soil, poor soil structure, and low available plant nutrients and reduce soil fauna-flora distribution [1]. Being these problems are continued, there are no sufficient studies conducted particularly on the effects of seed inoculation and vermicompost application on soil microbial communities and selected chemical properties. Therefore, this study was aimed to investigate the effects of Rhizobium strain and vermicompost rate on residual soil properties under greenhouse conditions.

2. Materials and Methods

The experiment was conducted from October 2020 to May 2021 under greenhouse conditions at Wollo University. The experimental soil was collected from Gerado kebele in Dessie Zuria District. Gerado kebele is geographically located at 11°15' 0"N of latitude and 39 ° 24' 0" E of longitude with ranges of altitude 2200 and 2800 meters above sea level.

The experiment was conducted in pot having the size of 40 cm in diameter and 35 cm height made of plastic locally named as *Baledi* which holds 20 liters water. Each pot had comparatively 1.256×10^{-5} ha in area coverage. Water requirement of the crop was supplied uniformly from drip irrigation. The experimental soil was *Vertisols* and characterized by high clay content, slightly dark in color and sticky character when wet [7, 22]. Vermicompost was fully decomposed by earth worms.

A fast growing *Rhizobium*, *leguminosarum biovar viceae* was used as bio-fertilizer. The *Rhizobium* strains (FB-1035 and FB-17) that released from HARC soil microbiology laboratory were used for seed inoculation at 500 g/ha rate. These strains have been proven to enhance the nodulation capacity, agronomic, and yield performance of faba bean under wide ecological conditions [1, 39].

2.1. Treatments, Experimental Procedures, and Design

The factorial combinations of four rates of vermicompost (0, 5, 10 and 15 ton/ha) and three levels of Rhizobium strain (non-inoculated, inoculated with RS-17 and RS-1035 strains) treatments were laid out in CRD and replicated three times (*Table 1*). A total of 36 pots were arranged in CRD with 4.5 m by 2 m (9 m^2) area in greenhouse. The space between replications and treatments was 0.5 m and 0.2 m, respectively and the pots were arranged in three rows and then five faba bean seeds planted in each pot at 8 cm depth on December 5, 2020.

The predetermined rates of vermicompost were incorporated into soil before one month of the crop sowing day. Vermicompost applications were rated on a dry-weight basis. Rhizobium inoculation was carried out during crop planting. At sowing, faba bean seeds were inoculated with predetermined rhizobium strain using sugar solution (10 g) for 100 ml of water as an adhesive agent and then, left to dry in the shade for minutes before planting as per [13] procedure. Weeds were controlled manually and all other remaining agronomic practices were properly practiced at given schedule. The crop was allowed growing for five months until the crop matured.

Table 1. *Treatment combinations of Rhizobium strains and vermicompost rate.*

Treatment number	Treatment combination	Treatment description
T1	RS_{0+} V_0	RS_0=un inoculated, V_0=0
T2	$RS_{0+}V_5$	RS_0= un inoculated, V_5=5
T3	$RS_{0+}V_{10}$	RS_0= un inoculated, V_{10}=10
T4	$RS_{0+}V_{15}$	RS_0= un inoculated, V_{15}=15
T5	RS-1035+V_0	RS-$_{1035}$= inoculated, V_0=0
T6	RS-1035+V_5	RS-$_{1035}$= inoculated, V_5=5
T7	RS-1035+V_{10}	RS-$_{1035}$= inoculated, V_{10}=10
T8	RS-1035+V_{15}	RS-$_{1035}$= inoculated, V_{15}=15
T9	RS-17+V_0	RS-$_{17}$= inoculated, V_0=0
T10	RS-17+V_5	RS-$_{17}$= inoculated, V_5=5
T11	RS-17+V_{10}	RS-$_{17}$= inoculated, V_{10}=10
T12	RS-17+V_{15}	RS-$_{17}$= inoculated, V_{15}=15

RS_0=Un-inoculated, RS-1035=Inoculated, RS-17=Inoculated, V_0=0, V_5=5 t/ha, V_{10}=10 t/ha and V_{15}=15 t/ha.

2.2. Soil Sampling and Analysis

Soil samples were collected from 36 total pots from a depth of 0-30cm using sterilized small garden shovel for soil microbial and selected chemical properties analysis. Soil bulk density was estimated using core sampler, which after drying the soil core samples to constant weight in an oven at 105°C for 24 hours as per the procedures described [9]. Soil texture was analyzed by the hydrometer method following the procedure described [12]. Soil pH was determined from the filtered suspension of 1:2.5 soils to water ratio using a glass electrode attached to a digital pH meter [17, 28]. Organic carbon and total nitrogen were determined by the method [38], and Kjeldhal methods [19], respectively. Available P was determined by extraction with 0.5 M $NaHCO_3$ according to the methods [26]. CEC was measured after saturating the soil with 1N ammonium acetate then displaying it with 1N sodium acetate [34]. Finally, 200g soils were collected from each experimental pot then prepared for the analysis of soil pH, organic carbon, total N, available P and CEC.

2.3. The Physicochemical Analysis of the Experimental Vermicompost

The organic wastes such as crop residues, weeds and cattle manure was decomposed by *Eisenia foetida* earth worm for three months to produce vermicompost that was used in this experiment. Its total nitrogen was determined using the Kjeldahl method as described by the research [19]. Its pH was estimated from the filtered suspension of 1:2.5 soils to water ratio while available P of the vermicopost was determined using Olsen's method [26] and organic carbon was estimated by using [38] procedure. Its CEC was determined by using [34] method which measures the CEC of the soil as displaced amount of Na^+ by ammonium acetate (NH_4OAc). The result of the soil analysis was detailed in Table 2.

Table 2. *The experimental soil and vermicompost physicochemical properties.*

Parameters	Soil Value	V.compost Value
Sand (%)	61	
Silt (%)	16	
Clay (%)	23	
Textural Class	Sandy clay loam	
BD (gcm^{-3})	1.5	
pH	6.39	7.02
Organic matter (%)	3.623	18.11
Organic carbon (%)	2.1	10.5
Total nitrogen (%)	0.15	0.9
Available P (ppm)	5.83	13.83
CEC (Meq/100g)	23.56	57.3

CEC=Cation Exchange Capacity, BD=Bulk Density, P=phosphorus, ppm= parts per million, Based on [6, 33].

2.4. Soil Microbial Community Parameters

Determination of Microbial Density: The experimental soil sample was collected from 36 pots and subjected for microbial load count at Wollo University, Biotechnology laboratory. One gram of soil sample was put in to a tube containing 9 ml of sterilized distilled water. After homogenizing the solution sterilized distilled water were added until the volume reaches to 10 ml and then tenfold serial dilution in sterile distilled water were performed up to 10^{-6} [8]. Subsequently, 0.1 ml were removed from 10^{-5} and 10^{-6} dilutions and inoculated in to standard plate count agar (nutrient agar) to determine the main representative total plate counts [25]. The plates were incubated at 37°C for 48 h and the total number of viable bacteria was calculated by the formula:

$$\text{Total bacteria/g} = \frac{\text{Number of Colonies x Dilution Factor}}{0.1ml(\text{Volume of inoculum})}$$

The results were expressed as the number of colony-forming units per gram (weight) of intestinal content (CFU/g).

Determination of Rhizobium Density: One grams of soil was taken as soil sample from each 36 experimental pots and suspended in 9 ml of sterile distilled water and shaken for 20 min. Ten-fold serial dilutions was made at scale of 1×10^{-3} and 1×10^{-4} [29]. Rhizobium bacteria were quantified on composition media composed of Manitol agar (10g 10^{-3} ml), K_2HPO4 (0.5g 10^{-3} ml), $MgSO_4$, $7H_2O$ (0.2g 10^{-3} ml), NaCl (0.1g 10^{-3} ml), yeast extract (0.5g 10^{-3} ml) and agar (7.5g 10^{-3} ml). After colony formed Rhizobia colony count was conducted for each 36 soil sample (containing 1g of soil) [36].

Rhizobium isolation and colony morphology characterization: after rhizobium bacteria count completed, two viable colonies of rhizobium bacteria was sub cultured in to pure culture-media (composition media), five media for each treatment (60 total composition media) [25]. Then, inoculated and sub-cultured media were incubated at 37°C for 72 hours. Finally, size, margin, opacity, shape and color of viable colonies were determined for each 12 treatments [36].

$$\text{Total rhizobium bacteria/g} = \frac{\text{Number of Colonies x Dilution Factor}}{0.1ml(\text{Volume of inoculum})}$$

The results were expressed as the number of colony-forming units per gram (weight) of intestinal content (CFU/g).

2.5. Data Analysis

The data collected from the experiment at different growth stages were subjected to statistical analysis (ANOVA) as per the experimental design using SAS version 9.4 and GenStat 12th edition. The mean separation was carried out using the least significant difference (LSD) test at $P\leq 0.05$. Interpretations were made following the procedure described [15].

3. Results and Discussions

3.1. Effect of Rhizobium Strains and Vermicompost Rate on Residual Soil Microbial Community

3.1.1. Effect on Soil Microbial Density

The number of bacteria was significantly ($P\leq 0.05$) affected by the main effect of inoculants strain and vermicompost rate whereas the interaction effects were insignificant. The maximum number of bacteria (1.33×10^8 cfu/g) was obtained from the highest rate of vermicompost while the minimum (1.08×10^8 cfu/g) was observed from lower rates of vermicompost (*Table 3*). Seed inoculation of rhizobium bacteria with RS-17 strain gave maximum number of bacteria (1.3×10^8 cfu/g) but it had statistical parity results with RS-1035 (1.2×10^8 cfu/g); however, the smallest number of bacteria was recorded from control pot (1.1×10^8 cfu/g) (*Table 3*). The increment in soil microbial population with application of vermicompost might be due to earthworms ingest plant growth-promoting rhizospheric bacteria such as pseudomonas, rhizobium, bacillus, azosprillium, azotobacter, etc. along with rhizospheric soil, and they might get activated or increased due to the ideal micro-environment of the gut. Hence, these earthworm activities attributed vermicompost to be rich in microbial populations and diversity, particularly bacteria and actinomycetes.

In agreement with this result [23] reported that the total microbial population and activity significantly increased by sole and combined application of (5 ton/ha) vermicompst with half dose of NPK over control treatment. The author [27] found that microbial numbers and their extracellular enzyme profiles were more abundant in vermicompost produced from fruit pulp, vegetable waste, groundnut husk and cow dung compared to the normal compost of the same parental origin. Also, the author [18] reported that application of maximum rates of vermicompost (40 ton/ha) produced the highest number of aerobic mesophilic bacterial (8.86×10^6 cfu/ g soil) than the same amount of (40 ton/ha) FYM treatments (5.92×10^6 cfu/ g soil) and control (1.98×10^6 cfu/ g soil). On other hand, [40] reported that faba bean inoculated with rhizobium significantly increased the number of clones of actinobacteria, acidobacteria and planctomycete after 16S rDNA bacteria clones were analyzed by ARDRA.

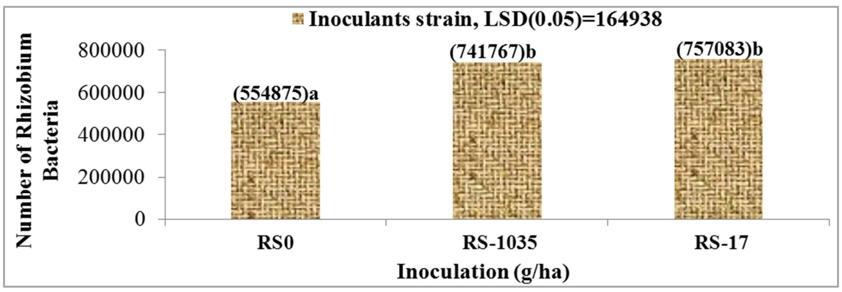
Table 3. *Main effect of inoculation and vermicompost rate on total number bacteria.*

Vermicompost rate (ton/ha)	Total number of Bacteria (cfu/g)
0	1.20×10^{8}ab
5	1.08×10^{8}a
10	1.24×10^{8}b
15	1.33×10^{8}b
CV (%)	10.9
LSD (0.05)	1.3×10^7
Rhizobium inoculants	Total number of Bacteria (cfu/g)
RS0	1.1×10^{8}a
RS-1035	1.23×10^{8}b
RS-17	1.3×10^{8}b
LSD (0.05)	1.1×10^7
LSD (0.05) = Least Significant Difference at 5%; and CV (%) =Coefficient of Variation	
Means with the same letter (s) are not significantly different at 5% level	

3.1.2. Effect on Rhizobium Bacteria Density

The number of rhizobium bacteria was significantly ($P\leq0.05$) affected by the main effect of inoculants strain but not significant influenced by vermicompost and their interaction effects. A pot treated with RS-1035 strain produced maximum number of rhizobium bacteria (757083cfu/g) which was statistically similar with pot treated with RS-17 strain (741767cfu/g) while the smallest number of rhizobium bacteria was obtained at the control (554875 cfu /g) pot (*Figure 1*). The possible reason might be indigenous microbial populations in the rhizosphere of faba bean enhanced competitiveness for nodule occupancy constructed by introducing an extra copy of a modified proline dehydrogenase gene when faba bean plant inoculated with *rhizobium,* RS-1035 and RS-17 strains. Also, it might be due to the dispersal of rhizobium bacteria through the soil from the colonization roots after nodulation of faba bean completed consequently large number of rhizobium bacteria accumulated in the soil.

This finding is in agreement with that of Trabelsi. D and Mhamdi. R [35] who reported that mono and dual soil inoculation with *Rhizobium gallicum* strain 8a3 and *Ensifer meliloti* strain 4H41 significantly increased bacterial communities, particularly PGPMs, such as *Rahnella*, *Bacillus*, *Azospirillum*, *Mesorhizobium, Pseudomonas*, *Streptomyces,* and *Sinorhizobium*. The researcher [40] also reported that faba bean seed inoculation with NGB-FR 62, 126, and 128 strains increased Rhizobia population by 6.92×10^7 c.f.u. g^{-1}, phosphate solubilizing bacteria by 4.47×10^7 c.f.u. g^{-1} and Actinomycetes by 2.40×10^7 c.f.u. g^{-1}.



Labels in the same designs followed by the same letter are not significantly different at p≤ 0.05.

Figure 1. *Number of Rhizobia population affected by the main effect of inoculations.*

3.1.3. Effect on Colony of Rhizobium

The colonies were having sticky appearance showing the production of mucous at lower levels of growing media. The bacteria turned the color of composition media (deep red) into slightly red color which is characteristic feature of acid producing and fast growing rhizobium bacteria. The colony transparency was opaque with entire margin and raised elevation observed on composition plain media. Microscopic view of rhizobium isolate showed that the isolates had spherical shape and smooth surface. Analysis of colony morphology indicated that the colony diameter ranged between 0.9-3.6 mm. Pots treated with T_4, T_5, T_6, T_8, T_9, T_{10}, T_{11} and T_{12} produced large sized rhizobia isolates while smallest sized rhizobium isolates were obtained at control treatments (*Table 4*). In agreement with this result, [3] reported that 95% of isolates of *R. leguminosarum* fall in colony size between 2-4 mm in diameter. The production of large sized and fast growing rhizobium bacteria with the application of vermicompost, rhizobium bacteria and their interaction might be attributed to rhizobia ability to withstand environmental stresses (temperature, salinity, and pH fluctuations in the soil) could be suitable to increase crop production as well as the development of commercial inoculants.

Table 4. *Morphological characteristics of rhizobium isolates.*

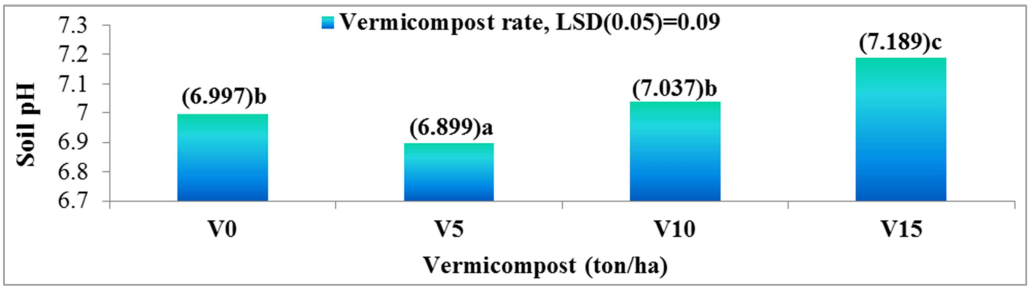
Treatment	Isolation code	Diameter (mm)	Size	Margin	Elevation	Opacity	Shape	Color
T_1 (Control)	T_1 $I_1.I_5$	1	small	Entire	Raised	Opaque	Spherical	Reddish
T_2 ($RS_{(Nil)}$+V_5)	T2 I_1-I_5	1.3	medium	Entire	Raised	Opaque	Spherical	Reddish
T_3 ($RS_{(Nil)}$+V_{10})	T3 I_1-I_5	1.6	medium	Entire	Raised	Opaque	Spherical	Reddish
T_4 ($RS_{(Nil)}$+V_{15})	T_4 I_1-I_5	2.3	Large	Entire	Raised	Opaque	Spherical	Reddish
T_5 (RS_{-1035}+V_0)	T_5 I_1-I_5	2.7	Large	Entire	Raised	Opaque	Spherical	Reddish
T_6 (RS_{-1035}+V_5)	T_6 I_1-I_5	2.2	Large	Entire	Raised	Opaque	Spherical	Reddish
T_7 (RS_{-1035}+V_{10})	T_7 I_1-I_5	1.9	medium	Entire	Raised	Opaque	Spherical	Reddish
T_8 (RS_{-1035}+V_{15})	T_8 I_1-I_5	2.1	Large	Entire	Raised	Opaque	Spherical	Reddish
T_9 (RS_{-17}+V_0)	T_9 I_1-I_5	2.9	Large	Entire	Raised	Opaque	Spherical	Reddish
T_{10} (RS_{-17}+V_5)	T_{10} I_1-I_5	2.3	Large	Entire	Raised	Opaque	Spherical	Reddish
T_{11} (RS_{-17}+V_{10})	T_{11} I_1-I_5	2.5	Large	Entire	Raised	Opaque	Spherical	Reddish
T_{12} (RS_{-17}+V_{15})	T_{12} I_1-I_5	2.3	Large	Entire	Raised	Opaque	Spherical	Reddish

The measured (the diameter of colony) value of isolated colonies characterized as; Large (>2 mm), measured value of the larger colonies ranges from 2-3.8 mm in diameter. Medium (1.2-2 mm), Small (<1.2mm) [20].

3.2. Effect of Rhizobium Strains and Vermicompost Rate on Selected Soil Chemical Properties

3.2.1. Effect on Soil pH

Soil pH was highly significantly ($P \leq 0.01$) affected by the main effect of vermicompost rate while significantly ($P \leq 0.05$) influenced by interaction of vermicompost and inoculants strain. The maximum soil pH was recorded in pot treated with 15 ton/ha vermicompost (7.19) while the lowest soil pH (6.90) was recorded in pot treated with 5 ton/ha vermicompost (Figure 2).



Labels in the same designs followed by the same letter are not significantly different at P≤ 0.05.

Figure 2. *Soil pH affected by the main effect of vermicompost application rate.*

On the other hand, the maximum soil pH (7.2) was recorded in pot receiving 15 ton/ha vermicompost with RS-17 stain but it had statistical parity results with V_{15}+RS_0, V_0+RS-17, V_{15}+RS-1035, V_{10}+RS_0, V_{10}+RS-103 and V_0+RS-1035, 7.2, 7.167, 7.167, 7.09, 7.06 and 7.047, respectively, although the control treatment had the lowest soil pH, it was at pare with pots that received 5 ton/ha vermicompost at all level of Rhizobium strains (*Table 5*). The rise in soil pH with application of vermicompost might be attributed by the secretion of $NH4^+$ ions that reduce the pool of H^+ ions release of anions to the soil and the activity of calciferous glands in earthworms containing carbonic anhydrase that catalyzes the fixation of CO_2 as $CaCO_3$, thereby preventing the fall in pH.

Study carried out by Bambara [5] showed that Rhizobium inoculation with molybdenum significantly increased the soil pH and the availability of Ca, Na, Fe, Cu, Zn and Mn in the rhizosphere soil. Getachew. A et al. [14] reported that combined application of 8 ton/ha FYM with P fertilizer significantly increases soil pH levels and nutrient concentrations in soil over control. Also, Ilker. U and Ismail. E. T. [18] stated that introduction of organic materials (30 ton/ha vermicompost and 30 ton/ha FYM) in to the soil significantly ($P \leq 0.01$) improved soil pH values 8.14 and 8.07, respectively over (7.96) untreated trail. In contrary with this result, the finding [4] showed that plots treated with vermicompost at the rate of 15 ton/ha had significantly reduced soil pH (7.33) as compared to control (8.0) plot. Likewise, the author [10] stated that, soil treated with maximum concentrations of pig manure vermicompost significantly reduced soil pH (5.68) than the control (6) treatments.

Table 5. *The interaction effect of inoculation and vermicompost rate on soil pH.*

Vermicompost rate (ton/ha)	Rhizobium strain		
	RS_0	RS-1035	RS-17
0	6.777^{a}	7.047^{cde}	7.167^{e}
5	6.917^{abc}	6.930^{abc}	6.850^{ab}
10	7.090^{de}	7.060^{cde}	6.960^{bcd}
15	7.200^{e}	7.167^{e}	7.200^{e}
CV (%)	1.3		
LSD (0.05)	0.16		

LSD (0.05) = Least Significant Difference at 5%; and CV (%) =Coefficient of Variation
Means with the same letter (s) are not significantly different at 5% level

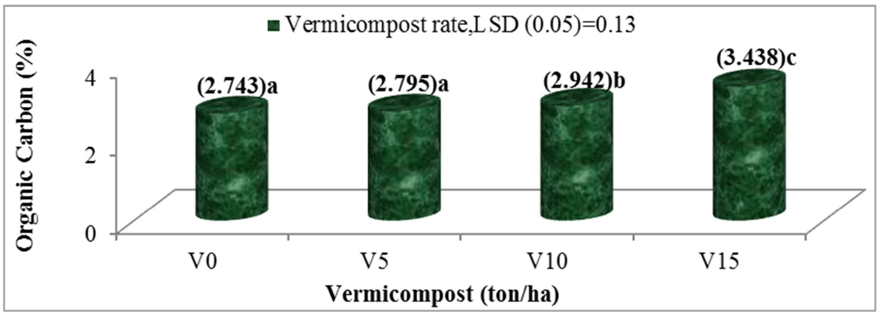
3.2.2. Effect on Soil Organic Carbon

Soil organic carbon was highly significantly (P≤ 0.01) affected by the main effect of vermicompost rate but significantly (P≤ 0.05) influenced by their interaction effect. There were no significant differences on soil organic carbon due to the main effect of Rhizobium strains. Considering the main effect of vermicompost; the highest organic carbon (3.438%) was recorded on the soil treated by 15 ton/ha vermicompost but the lowest soil organic carbon (2.743%) obtained from control pot (*Figure 3*).

Table 6. *Effect of inoculation and vermicompost rate interaction on soil organic carbon (%).*

Vermicompost rate (ton/ha)	Rhizobium strain		
	RS_0	RS-1035	RS-17
0	2.529^{a}	2.776^{bc}	2.923^{bcd}
5	2.794^{bcd}	2.865^{bcd}	2.728^{ab}
10	2.982^{cd}	2.833^{bcd}	3.012^{d}
15	3.461^{e}	3.376^{e}	3.478^{e}
CV (%)	4.5		
LSD (0.05)	0.23		

LSD (0.05) = Least Significant Difference at 5%; and CV (%) =Coefficient of Variation
Means with the same letter (s) are not significantly different at 5% level



Labels in the same designs followed by the same letter are not significantly different at P≤ 0.05.

Figure 3. *Main effect of vermicompost rate on soil organic carbon.*

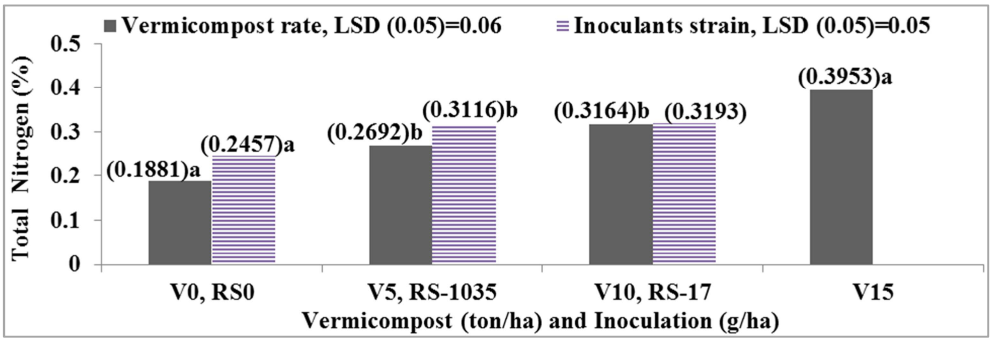
Considering the interaction effect; soil treated by using 15 ton/ha vermicompost rate with RS-17 strains resulted the highest organic carbon (3.478%) which was also at pare with V_{15}+RS_0 and V_{15}+RS-1035 and the lowest soil organic carbon (2.529%) recorded from untreated pot (*Table 6*). In line with this result [11] reported that soil treated with 10 ton/ha FYM with nitrogen fixer-A and phosphate solubilizing bacteria gave the highest organic matter content (12.5g/ka) than control (10.1g/kg) treatment. Similarly, [31] found that soil organic carbon content (12.8g/kg) increased significantly in the plots that had conjointly received FYM and inoculation of seed with Rhizobium than plots that had fertilized with chemical fertilizers alone (8.2g/kg) and control (8.1g/kg) plots. [18] found that increasing the rates of organic materials (40 ton/ha vermicompost and 40 ton/ha compost) highly significant (P≤ 0.01) increased soil organic matter content (3.1% and 3.0%, respectively) over (1.92%) control. Karmakar. S et al. [21] also reported that sole application of 100% vermicompost and 100% FYM significantly increased organic matter content (0.97%) and (0.94%), respectively than 100% chemical fertilizer (0.59%) and untreated (0.41%) plots.

3.2.3. Effect on Soil Total Nitrogen

Soil total nitrogen was highly significantly (P≤ 0.01) affected by the main effect of vermicompost rate but significantly (P≤ 0.05) influenced by the individual effect of rhizobium inoculants and their interaction effect. The highest soil total nitrogen (0.3953%) was observed in the pots received 15 ton/ha vermicompost while the lowest soil total nitrogen (0.1881%) was recorded from untreated pot. Faba bean inoculation with RS-17 strain gave the highest soil total nitrogen (0.3193%) but the lowest soil total nitrogen (0.2457%) was recorded from un-inoculated pot (*Figure 4*).

Considering their interaction effect; soils treated with 15 ton/ha vermicompost in combination with RS-17 strain gave the highest soil total nitrogen (0.4151%) while the lowest soil total nitrogen (0.1495 %) was observed from untreated pot (*Table 7*). The improvement of soil total nitrogen with application of vermicompost and rhizobium inoculations might be due to the symbiotic relationship between *Rhizobium* and faba bean plants, which result in fixation of atmospheric N into the roots and leading to increases in soil total nitrogen accumulation in faba bean rhizosphere soil. This result agreed with the result [31] which confirmed that

Rhizobium inoculated seeds in conjunction with 5 ton/ha FYM significantly increased available nitrogen contents (380 kg/ha) over (275 kg/ha) absolute control treatment. Hasan, M. [16] found that Rhizobium inoculation combined with phosphorus fertilizer increased soil total nitrogen (0.28%) than individual application of N and P fertilizers (0.2 and 0.17%, respectively) as well as control (0.16%) treatments. Belay. Z and Assefa. F. [8] also reported that faba bean inoculated with AUFR128 and AUFR115 isolated strains significantly increased soil total nitrogen (2.9%) and (2.8%), respectively than untreated plots. The researcher [4] stated that soils treated with sheep manure vermicompost at the rate of 15 ton/ ha had more total N compared to soils without vermicompost application.



Labels in the same designs followed by the same letter are not significantly different at P≤ 0.05.

Figure 4. *Total N as influenced by the main effect of vermicompost rate and inoculations.*

Table 7. *Effect of inoculants strain and vermicompost rate interaction on total N (%).*

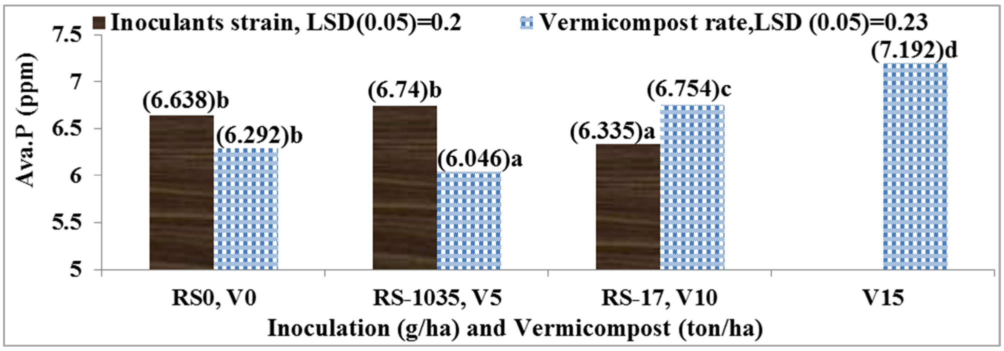
Vermicompost rate (ton/ha)	Rhizobium strain RS$_0$	RS-1035	RS-17
0	0.1495^{a}	0.4045^{e}	0.3952^{e}
5	0.1845^{ab}	0.1947^{abc}	0.1850^{ab}
10	0.2405^{abc}	0.2851^{cd}	0.2821^{bcd}
15	0.4085^{e}	0.3622^{de}	0.4151^{e}
CV (%)	20		
LSD (0.05)	0.099		

LSD (0.05) = Least Significant Difference at 5%; and CV (%) =Coefficient of Variation

Means with the same letter (s) are not significantly different at 5% level

3.2.4. Effect on Soil Available Phosphorus

The available phosphorus was highly significantly (P≤ 0.01) affected by the main effect of vermicompost rate but significantly (P≤ 0.05) influenced by the individual effect of rhizobium inoculants and their interaction effect. The highest available P (7.192 ppm) was observed in the pots treated with 15 ton/ha vermicompost rate while the lowest available P (6.046 ppm) was obtained from the pots treated with 5 ton/ha vermcompost. Rhizobium inoculation with RS-1035 strain gave the highest available P (6.740 ppm) but the lowest available P (6.335 ppm) was recorded from inoculation with RS-17 strain (*Figure 5*).



Labels in the same designs followed by the same letter are not significantly different at P≤ 0.05.

Figure 5. *Main effect of inoculants strain and vermicompost rate on soil Ava.P.*

Considering their interaction effects; combined use of 15 ton/ha vermicompost and Rhizobium inoculation with RS-1035 resulted the highest available P (7.333 pmm) while the lowest available P (5.908 pmm) was recorded at control pot (*Table 8*). The enhancement of available P with maximum application of vermicompost and Rhizobium inoculation might be due to the continuous inputs of P to the soil from slow release from vermicompost and release of P was due largely to the activity of soil microorganisms. This result agrees with the result reported by [14] who stated that combined application of 8 ton/ha vermicompost with chemical P significantly (P≤ 0.01) increased available P (6.4 ppm) over the (4.2 ppm) control. Similarly, [21] reported that individual application of 100% vermicompost and 100% FYM significantly increased available P (48.04 kg/ha) and (46.73kg/ha), respectively as compared to chemical fertilizer (41.07kg/ha) and control (38.06 kg/ha) treatments. Azarmi. R. et al. [4] also disclosed that soil treated by vermicompost at the rate of 15 ton/ha significantly (P≤ 0.05) increased available P (18.733 ppm) as compared to control plot (5.9 ppm).

Table 8. *The interaction effect of inoculation and vermicompost rate on soil Ava.P (pmm).*

Vermicompost rate	Rhizobium strain		
(ton/ha)	RS_0	RS-1035	RS-17
0	5.908[a]	6.927[de]	6.040[a]
5	6.253[ab]	5.957[a]	5.927[a]
10	7.060[def]	6.743[cd]	6.460[bc]
15	7.330[ef]	7.333[f]	6.913[d]
CV (%)	3.7		
LSD (0.05)	0.41		

LSD (0.05) = Least Significant Difference at 5%; and CV (%) =Coefficient of Variation
Means with the same letter (s) are not significantly different at 5% level.

3.2.5. Effect on Soil Cation Exchange Capacity

The cation exchange capacity was highly significantly (P≤ 0.01) affected by the main effect of vermicompost rate and their interaction effect but insignificantly (P≥ 0.05) influenced by rhizobium inoculations. The highest CEC (26.61 meq/100) was recorded from the pot received 15 ton/ha vermicompost while the lowest CEC (24.38 meq/100) was recorded from the pot treated with lower vermicompost rate (*Figure 6*).

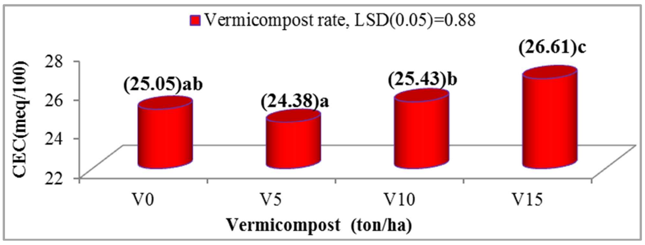


Figure 6. *CEC as influenced by the main effect of vermicompost application rate.*

Labels in the same designs followed by the same letter are not significantly different at P≤ 0.05.

Table 9. *The interaction effect of inoculants strain and vermicompost rate on CEC of soil (meq/100).*

Vermicompost rate	Rhizobium strain		
(ton/ha)	RS_0	RS-1035	RS-17
0	22.65[a]	25.59[cd]	26.90[de]
5	24.69[bc]	24.65[bc]	23.80[ab]
10	26.24[de]	25.69[cde]	24.35[bc]
15	26.99[de]	27.17[e]	25.65[cd]
CV (%)	3.5		
LSD (0.05)	1.5		

LSD (0.05) = Least Significant Difference at 5%; and CV (%) =Coefficient of Variation
Means with the same letter (s) are not significantly different at 5% level.

From their interaction effect; the highest CEC (27.17 meq/100) was recorded from the pots treated with 15 ton/ha vermicompost and Rhizobium inoculants with RS-1035 strain but the lowest CEC (22.65 meq/100) obtained from untreated pot (*Table 9*). The increment of CEC with rate of vermicompost and Rhizobium inoculation might be due to release of cations with the decomposition of vermicompost which would have increased the CEC. This is in line with the observation of [11] who reported that soil treated by combined application of 5 ton/ha FYM with inoculation and half dose of chemical fertilizer gave the highest cation exchange capacity (13.7cmol/kg) over control (11.9 Cmol/kg) treatment. Hasan, M. [16] stated that *Rhizobium* inoculation combined to N and P fertilizer significantly increased soil CEC (7 meq/100) over control (2.03 meq/100) treatment. Broz. A. P. et al. [10] reported that application of vermicopmost increased the cation exchange capacity with values ranging from 40-300 cmol kg^{-1}. Also, Manivannan. S et al. [23] found that sole and combined application of 5 ton/ha vermicompost with 50% NPK fertilizer significantly increases CEC (28.7cmol/kg) and (26.90cmol/kg) respectively over (24.30 cmol/kg) control.

4. Conclusions

Sole application of (15 ton/ha) vermicompost and Rhizobium strains; RS-17 or RS-1035 significantly improve the number of microbial population and rhizobia population, than control and minimum application vermicompost. Whereas, the main effect of Rhizobium strains were predominantly increased number of Rhizobia population in the soil. Sole and combined application of 15 ton/ha vermicompost with RS-17 and RS-1035 strains produced large sized, deep red and fast growing rhizobium bacteria.

On the other hand, integrated use of 15 ton/ha vermicompost with RS-1035 and RS-17 strains significantly improved soil pH, organic carbon, total nitrogen, available P and cation exchange capacity. Therefore, it is recommended that integrated application of 15 ton/ha vermicompost and 500g/ha Rhizobium inoculants with RS-17 strain was prominent to increase yield of faba bean, enhance beneficial microbial communities and improve soil fertility in Dessie Zuria district. However, this experiment conducted at green house, so it should be repeated on field condition to make a conclusive recommendation.

Declarations

I declare and confirm that the study entitled "Effect of Rhizobium Strains and Vermicompost Rate on Residual Soil Properties under Greenhouse Condition" is my work. I have followed all required ethical principles.

Conflicts of Interest

The author declares that there has no conflict of interest.

Data Availability

The data used to support the results of this study are available from the corresponding author upon request.

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